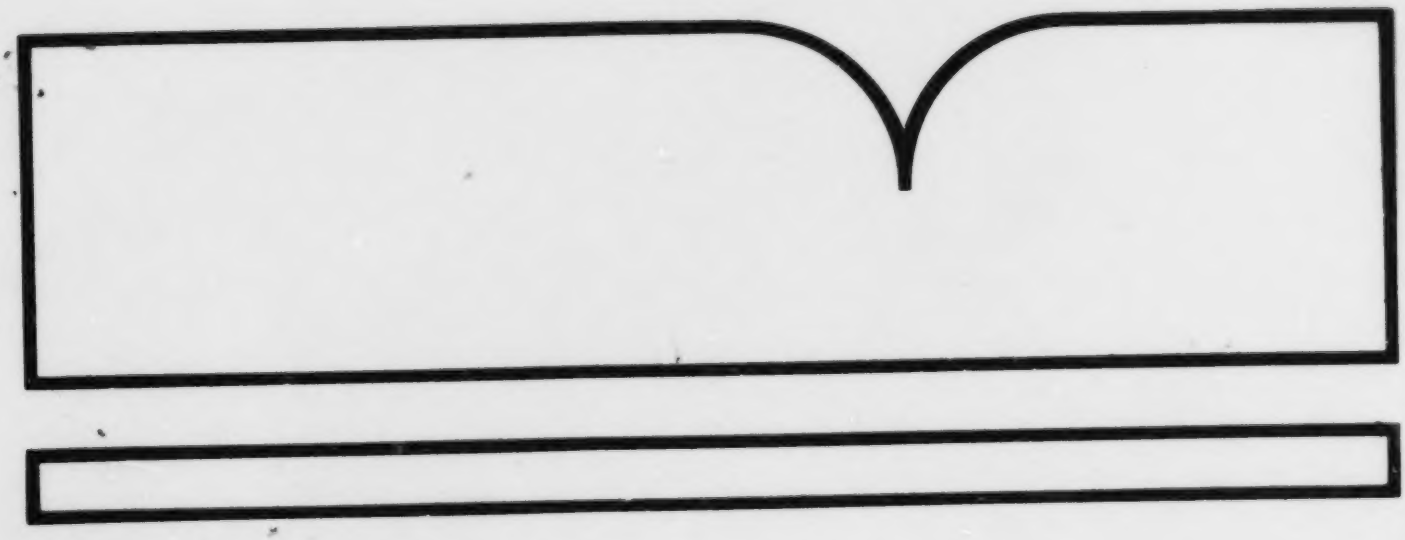
PB84-175272

Comparative Renal and Hepatotoxicity of Halomethanes: Bromodichloromethane, Bromoform Chloroform, Dibromochloromethane and Methylene Chloride

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1983



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA-600/J-83-199	2.	3 PB84-175272
4. TITLE AND SUBTITLE Subchronic Toxicology of Halomethanes: Bromodichloromethane, Bromoform, Chloroform, Dibromochloromethane and Methylene Chloride (Journal Version)		5. REPORT DATE 1983 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Lyman W. Condie, Carolyn L. Smallwood, R. Dana Laurie		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Target Organ Toxicology Branch Toxicology & Microbiology Division Health Effects Research Laboratory U.S. Environmental Protection Agency Cincinnati, OH 45268		10. PROGRAM ELEMENT NO. AAQBIA 11. CONTRACT/GRANT NO. (in-house)
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research & Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600-11

15. SUPPLEMENTARY NOTES

REF: Drug and Chemical Toxicology, 6(6):563-578, 1983

16. ABSTRACT

The subchronic renal and hepatotoxicities of five selected halomethanes, which are drinking water contaminants, were evaluated following a 14-day exposure period. Bromodichloromethane, bromoform, chloroform, dibromochloromethane and methylene chloride were administered at three dose levels. Toxicity was evaluated by measuring increases in total body weight, active uptake of p-aminohippuric acid into renal cortical slices, blood urea nitrogen, serum creatinine and serum glutamate--pyruvate transaminase levels and by performing a histopathologic examination of liver and kidney tissues. At the dose level empolyed in these experiments, dose-related effects on the liver and kidney were seen with the uptake of p-aminohippuric acid into kidney slices and with the histopathologic evaluation of tissues. The other parameters revealed adverse renal and hepatic effects in only the high dose groups. Treatment-related effects seen in the methylene chloride exposed mice were less pronounced than in the other halomethane treatment groups. In general, histopathological changes were the most sensitive indicators of both liver and kidney damage.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) unclassified	21. NO. OF PAGES 17
	20. SECURITY CLASS (This page) unclassified	22. PRICE

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-83-199
JOURNAL ARTICLE

DRUG AND CHEMICAL TOXICOLOGY, 6(6), 563-578 (1983)

COMPARATIVE RENAL AND HEPATOTOXICITY OF HALOMETHANES: BROMODICHLOROMETHANE, BROMOFORM, CHLOROFORM, DIBROMOCHLOROMETHANE AND METHYLENE CHLORIDE

L. W. Condie, C. L. Smallwood, R. D. Laurie
Target Organ Toxicology Branch
Toxicology and Microbiology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Cincinnati, OH 45268

ABSTRACT

The renal and hepatotoxicities of five selected halomethanes, which are drinking water contaminants, were evaluated following a 14-day exposure. Bromodichloromethane, bromoform, chloroform, dibromochloromethane and methylene chloride were administered at three dose levels. Toxicity was evaluated by measuring changes in total body weight, uptake of p-aminohippurate into renal cortical slices, blood urea nitrogen, serum creatinine, and serum glutamate-pyruvate transaminase levels and by performing a histopathologic examination of liver and kidney tissues. Dose-related effects on the liver and kidney were detected with the uptake of p-aminohippurate into kidney slices and with the histopathologic evaluation of tissues. Treatment-related effects seen in the methylene chloride exposed mice were less pronounced as compared to the other halomethane treatment groups. In general, histopathological changes were the most sensitive indicators of both liver and kidney damage.

INTRODUCTION

Recent research studies have demonstrated that chlorine disinfection of drinking waters results in the formation of trihalomethanes, primarily chloroform.[1-3] Substantial concern has arisen because of the toxicological properties of some of these chemical substances.

While halogenated aliphatic hydrocarbon compounds have pronounced toxicological effects on various organs following acute industrial or accidental exposures,[4] the renal and hepatic effects of repeated oral exposures or chronic low level ingestion of halomethanes in drinking water are not as well documented. A study evaluating the subchronic and immunotoxicity of selected halomethanes which are drinking water contaminants has recently appeared.[5] The experiments presented in this paper were designed to further investigate the toxicity of certain halomethanes and to compare their effects on the liver and kidney. Specific clinical chemistry and biochemical tests, which are affected by the acute administration of the halomethanes, were utilized to detect renal and hepatic damage following a 14-day subchronic exposure.[6,7] The clinical and biochemical changes that indicated kidney and liver damage were confirmed by histopathological evaluation. Part of this work has been presented in abstract form.[8]

METHODS

Animal Handling and Exposure

Male CD-1 mice were purchased from Charles River (Portage, MI), held for one week quarantine period, and randomly housed, four per plastic cage with hardwood chip bedding (Absorb-Dri, St. Regis Paper, Garfield, NJ). Occasionally, an aggressive mouse would have to be placed in a single cage. The mice were individually marked by toe clipping. Purina Lab Chow and distilled water which had been carbon filtered and irradiated

with ultraviolet light were available ad libitum. The animal room was maintained at 20-22°C with 40-60% relative humidity and 12 hour interval light-dark cycles.

Technical grade chloroform (Fisher, Fairlawn, NJ), bromoform (Aldrich, Milwaukee, WI), bromodichloromethane, dibromochloromethane (Pfaltz and Bauer, Stamford, CT) and methylene chloride (Baker, Phillipsburg, NJ) were commercially purchased. The halomethanes were dissolved in corn oil, and various concentrations were administered by gavage in a total volume of 0.1 ml/30 g body weight for 14 consecutive days. The dose levels, determined by preliminary studies, are indicated in the tables. The administration of the compounds was staggered so that a maximum of 16 mice were killed on a given day (4 mice from each treatment group).

Biological Observations

Animals were observed daily for overt signs of toxicity. Individual body weights were obtained on days 1 and 14. Blood samples were collected from the inferior vena cava after cervical dislocation. The blood was allowed to clot, and the sera were frozen at -70°C to be utilized later for clinical chemistry studies. Blood urea nitrogen (BUN), serum creatinine, and serum glutamate-pyruvate transaminase (SGPT) were measured by a Union Carbide Centrifichem high speed centrifugal kinetic analyzer.

One kidney was removed and placed in ice-cold Krebs-Ringers solution. After renal cortical slices were prepared using a hand slicer, active transport of p-aminohippurate (PAH) into renal

cortical slices was determined by the method of Cross and Taggart.[9] Lactic acid (10mM) was added to the incubation medium to maximally stimulate slice uptake of PAH. The kidney slices were incubated for 90 minutes at 25°C. The other kidney and representative hepatic tissue were preserved in 10% buffered formalin for histopathologic evaluation. Based on the biochemical data, liver and kidney tissues were examined from ten of the animals in all of the effected dose groups and in the highest no adverse effect groups. Kidney and liver tissues from five of the animals in the other apparent no adverse effect groups were examined. The hematoxylin and eosin stained slides were evaluated by a board-certified veterinary pathologist, Dr. William Iverson of Experimental Pathology Laboratories, Herndon, VA.

Statistical Analyses

Experimental data, excluding the histopathology results, were entered into and analyzed by the Statistical Analyses System (SAS Institute, Cary, NC). One-way analysis of variance (ANOVA) for each parameter was conducted to test the hypothesis that there were response differences between the groups. When the ANOVA indicated an effect, Duncan's Multiple Range Test[10] was used to detect differences ($p < 0.05$) between treatment groups. The data and the indication of significant differences are presented in the tables.

RESULTS

The effect of halomethane administration on mouse body weight is shown in Table 1. There was a slight, but statistically

TABLE 1. Effect of Halomethanes on Mouse Body Weight

Compound	Dose (mg/kg)	Body Weight(g)* Day 1	Day 14
Bromodichloromethane	0	37.3 ± 0.7	39.3 ± 1.1
	37	38.7 ± 0.5	39.4 ± 0.5
	74	39.1 ± 0.9	40.3 ± 0.6
	148	39.1 ± 1.7	38.8 ± 1.7
Bromoform	0	31.2 ± 0.5	32.0 ± 0.6
	72	32.4 ± 0.4	33.2 ± 0.4
	145	31.2 ± 0.6	32.3 ± 0.8
	289	30.9 ± 0.4	32.6 ± 0.5
Chloroform	0	32.1 ± 1.0	34.0 ± 0.5
	37	33.5 ± 0.3	33.8 ± 0.4
	74	34.8 ± 0.7	35.2 ± 0.7
	148	32.1 ± 0.4	30.9 ± 0.8#
Dibromochloromethane	0	34.7 ± 1.1	34.6 ± 0.8
	37	34.5 ± 1.0	34.6 ± 0.8
	74	34.0 ± 0.8	34.2 ± 0.7
	147	35.0 ± 0.8	33.7 ± 0.8
Methylene Chloride	0	40.9 ± 1.4	42.4 ± 1.5
	133	38.5 ± 0.8	39.5 ± 1.0
	333	38.2 ± 0.9	39.0 ± 1.4
	665	38.4 ± 1.5	39.6 ± 1.7

*Each value represents the mean ± S.E.M. of at least 8 animals.

#Denotes significant difference from the other chloroform animal groups ($p < 0.05$).

significant, body weight loss (9%) in the chloroform high dose treatment group. The administration of the other halomethanes did not significantly effect mouse body weights.

The measurement of active uptake of the organic acid PAH into kidney slices served as an indicator of the functioning of the renal proximal tubles (Table 2). The renal slice uptake of PAH was significantly inhibited by 27% and 53% in the two highest bromodichloromethane treatment groups and was also significantly inhibited by 30% in the bromoform highest exposure group. A dose response relationship was seen in the chloroform exposed mice in which the active uptake of PAH was inhibited by 15% and 61% in the middle and high dose groups, respectively. In the dibromochloromethane exposed animals, the PAH uptake was only inhibited in the high dose group. The PAH uptake into renal cortical slices in each of the methylene chloride exposed groups of animals was inhibited to a similar degree.

As seen in Table 3, the clinical chemistry measurements for evaluating renal function, BUN and serum creatinine, were not as sensitive to the renal toxic effects of the halomethanes as were the PAH uptake measurements. None of the serum creatinine levels were elevated in any of the exposure groups while BUN levels were only significantly elevated in the chloroform high exposure group.

The analysis of the SGPT measurements revealed treatment related effects. SGPT values were elevated in the high exposure groups of all the compounds investigated except methylene chloride.

TABLE 2. Effect of Halomethanes on Renal Cortical Slice Uptake of PAH

Compound	Dose (mg/kg)	Slice/Medium Ratio*
Bromodichloromethane	0	18.3 ± 1.5[a]
	37	18.1 ± 0.9[a]
	74	13.4 ± 0.4[b]
	148	8.8 ± 0.3[c]
Bromoform	0	19.1 ± 1.5[a]
	72	18.5 ± 1.5[a]
	145	18.1 ± 1.3[a]
	289	13.3 ± 1.4[b]
Chloroform	0	14.8 ± 0.8[a]
	37	15.4 ± 0.8[a]
	74	12.6 ± 0.6[b]
	148	5.8 ± 0.6[c]
Dibromochloromethane	0	14.3 ± 0.8[a]
	37	12.5 ± 0.8[a,b]
	74	14.0 ± 0.9[a]
	147	10.4 ± 0.5[b]
Methylene Chloride	0	18.8 ± 2.0[a]
	133	14.6 ± 0.9[b]
	333	14.1 ± 0.6[b]
	665	14.3 ± 1.6[b]

*Each value represents the mean ± S.E.M. of at least 8 animals. Significant differences ($p < 0.05$) within a chemical treatment group are indicated by different letters.

TABLE 3. Effect of Exposure to Halomethanes on Selected Clinical Chemistry Parameters*

Compound	Dose (mg/kg)	BUN (mg-%)	Creatinine (mg-%)	SGPT (I.U./L)
Bromodichloro-methane	0	24.6 ± 1.0[a]	0.44 ± 0.03	20 ± 1[a]
	37	20.7 ± 1.1[b]	0.41 ± 0.03	26 ± 10[a]
	74	20.5 ± 0.7[b]	0.47 ± 0.02	21 ± 1[a]
	148	24.0 ± 0.8[a]	0.51 ± 0.04	51 ± 10[b]
Bromoform	0	22.9 ± 1.2	0.42 ± 0.05	21 ± 2[a]
	72	22.4 ± 0.7	0.46 ± 0.02	21 ± 2[a]
	145	23.9 ± 0.7	0.38 ± 0.03	17 ± 1[a]
	289	25.0 ± 1.9	0.42 ± 0.04	30 ± 4[b]
Chloroform	0	24.0 ± 1.6[a]	0.45 ± 0.07[a,b]	19 ± 3[a]
	37	21.9 ± 1.0[a]	0.40 ± 0.03[a,b]	17 ± 1[a]
	74	21.9 ± 0.9[a]	0.37 ± 0.02[b]	24 ± 4[a]
	148	37.1 ± 4.5[b]	0.52 ± 0.05[a]	102 ± 31[b]
Dibromochloro-methane	0	20.1 ± 3.2	0.49 ± 0.04	16 ± 1[a]
	37	24.6 ± 2.9	0.49 ± 0.03	16 ± 1[a]
	74	26.2 ± 4.5	0.55 ± 0.06	21 ± 2[a]
	147	25.2 ± 1.2	0.48 ± 0.05	35 ± 6[b]
Methylene Chloride	0	23.7 ± 1.1	0.45 ± 0.01	20 ± 2
	133	22.6 ± 1.2	0.41 ± 0.03	19 ± 4
	333	25.2 ± 1.7	0.45 ± 0.06	16 ± 1
	665	22.1 ± 1.0	0.44 ± 0.02	15 ± 1

*Significant differences ($p < 0.05$) within a compound treatment group are indicated by different letters.

A detailed listing of dose-related microscopic changes seen in the sections of kidney and liver are presented in Tables 4 and 5. The incidence and severity of lesions are summarized by treatment group for the respective chemical.

Bromodichloromethane produced intratubular mineralization, epithelial hyperplasia, and cytomegaly of tubular epithelial

TABLE 4. Incidence and Severity of Dose-Related Microscopic Changes of the Kidney from Exposure to Halomethanes

Treatment	Number of Animals Examined	Intra-tubular Mineral-ization	Epithe-lial Hyper-plasia	Cyto-megaly	Mesan-gial Hyper-trophy	Mesan-gial Neph-rosis
Severity of Change*		1234	1234	1234	1234	1234
Corn Oil	16		2			
Bromodichloro-methane						
37 mg/kg	9		1			
74 mg/kg	10	1	1	1		
148 mg/kg	10	2111	1521	51		
Bromoform						
72 mg/kg	5					
145 mg/kg	10		1		2	41
289 mg/kg	10	1	32		1	61
Chloroform						
37 mg/kg	10	2	11	21		
74 mg/kg	10	1	2	4		
148 mg/kg	10	4121	342	44		
Dibromochloro-methane						
37 mg/kg	5				4	
74 mg/kg	10				7	
147 mg/kg	10		211		43	
Methylene Chloride						
133 mg/kg	5					
333 mg/kg	5					
665 mg/kg	5					

*Key to severity Ratings: 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe/severe

TABLE 5. Incidence and Severity of Dose-Related Microscopic Changes of the Liver from Exposure to Halomethanes

Treatment	Number of Animals Examined	Centrilobular Pallor	Mitotic Figures	Focal Inflammation	Cytoplasmic Vacuolation
Severity of Change*		1234	1234	1234	1234
Corn Oil	16			1	1
Bromodichloromethane					
37 mg/kg	9	2		2	
74 mg/kg	10	621	132	4	
148 mg/kg	10	522	1	511	1
Bromoform					
72 mg/kg	5				
145 mg/kg	10	6	1	1	2
289 mg/kg	10	5111	1 1	33	1
Chloroform					
37 mg/kg	10	61	11	21	
74 mg/kg	10	811	421	2	
148 mg/kg	10	152	244	33	1
Dibromochloromethane					
37 mg/kg	5				
74 mg/kg	10		1 1	1	21
147 mg/kg	10		31	1	4 35
Methylene Chloride					
133 mg/kg	5			1	
333 mg/kg	5	1			3
665 mg/kg	5			1	31

*Key to severity Ratings: 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe/severe

cells in the majority of the animals of the high dose group. The intratubular mineralization and epithelial hyperplasia were moderate to moderately severe in some animals in this group. This chemical also produced centrilobular cytoplasmic pallor and focal inflammation of the liver in all treatment groups with increasing severity in a dose-related fashion. Mitotic figures, however, were most numerous in animals which had received the middle dose.

The administration of bromoform caused hyperplasia of tubular epithelial cells and enlargement (hypertrophy) and degenerative changes in the glomerular mesangium in both the middle and high dose treatment groups. The high dose of this compound produced frequent and severe changes in the liver, ranging from minimal to moderately severe centrilobular cytoplasmic pallor, minimal to slight focal inflammation, and increased mitotic figures in two animals. The centrilobular pallor consisted of a diffuse pale staining of the cytoplasm of hepatocytes, which were not enlarged, surrounding the central vein and extending to the midzonal region. Distinct vacuolation or necrosis was usually not a part of this lesion.

Microscopic changes that appeared to be related to the administration of chloroform to CD-1 mice included renal intratubular mineralization, epithelial hyperplasia and cytomegaly, which occurred in much greater frequency in the high dose group. In the liver, centrilobular cytoplasmic pallor, mitotic figures, and focal inflammation were treatment-related lesions. Mitotic fig-

ures, indicative of marked cell proliferation, were particularly striking in the high dose group.

Dibromochloromethane caused a treatment-related increase in the incidence of tubular epithelial hyperplasia and mesangial hypertrophy in the kidney. The occurrrence of mitotic figures and cytoplasmic vacuolation was increased in a dose-related fashion in the liver. In both of these tissues, however, the changes were usually only minimal to slight in severity.

The only histologic change that appeared to be related to methylene chloride administration was minimal to slight hepatic centrilobular cytoplasmic vacuolation.

DISCUSSION

This study was designed to examine and compare the effects of multiple exposure to selected halomethanes on the liver and kidney of exposed mice. One widely used criteria to detect toxicity from exposure to a chemical substance has been the reduction in the body weight gain in the exposed experimental animals.[11] Minimal effects on animal body weights were noted in all treatment groups except the high dose chloroform group.

Selected biochemical and clinical chemistry tests were employed to evaluate renal and hepatic damage. SGPT levels have been shown to be greatly elevated following acute chlorinated hydrocarbon exposure,[6] while the acute exposure of halogenated hydrocarbons inhibits the accumulation of organic ion uptake into renal cortical slices.[7] Four of the five halomethanes caused the SGPT levels to be significantly elevated in the high dose groups.

However, higher exposure levels must be employed before marked increase in SGPT levels are seen.[5] The clinical chemistry tests for kidney function, BUN and serum creatinine, were within the normal range following the halomethane exposures. When a more sensitive test was employed, impaired kidney function was detected. The in vitro accumulation of PAH was a more sensitive indicator of renal damage than were the measurements of BUN or serum creatinine. The in vitro finding confirms an earlier study by Kluwe[12] in which renal tests that measured functional capacities rather than unstressed, steady-state function were more sensitive to the effects of renal toxins. Clinical parameters which have been developed for the purpose of detecting acute liver or kidney damage may be of limited usefulness in judging renal or hepatic injury following low level subchronic or chronic exposure to chemicals. Insensitivity of the well known clinical parameters to detect low level chemical damage may be due to the large functional reserve and/or the repair capability of the liver and kidney or some other type of adjustment made by the exposed animal.

Treatment-related histological changes were detected in livers from animals exposed to each of the chemicals, though the changes were minimal in the dibromochloromethane and methylene chloride studies. Histopathological changes were seen in the glomerulus of the bromoform and dibromochloromethane exposed animals while proximal tubular changes were primarily detected in the bromodichloromethane and chloroform treated animals. This difference in structural renal damage between compounds is confirmed by the

experimental results presented in Table 2 that show a greater inhibition of PAH uptake into renal cortical slices in the bromodichloromethane and chloroform groups of mice. One indicator of proximal tubular damage is the inhibition of PAH uptake into renal cortical slices.

When comparing the relative toxicity of these halomethanes, the molecular weight difference must be considered. The biochemical and clinical data indicates that the relative toxicity of these compounds in order of decreasing toxicity is (1) chloroform, (2) dibromochloromethane, (3) bromodichloromethane, (4) bromoform, and (5) methylene chloride. The same order of toxicity is seen when comparing the histopathological changes except that the dibromochloromethane treated animals were the most sensitive to structural alterations following the 14 day chemical exposure.

While methylene chloride was not expected to be renal or hepatotoxic with the doses employed, it has been shown to be hepatotoxic in high doses.[13] Methylene chloride was included for comparison purposes since the experimental data will be particularly useful in determining doses for future experiments involving exposures of longer duration.

A multidisciplinary approach must be taken when determining the observable effect levels from exposure to chemicals detected in the environment since clinical parameters are often of limited value in assessing renal and hepatotoxicity. It appears necessary to examine both structural and functional changes in the assessment of renal toxicity and hepatotoxicity of pollutants detected in drinking water.

ACKNOWLEDGEMENTS

This article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsements or recommendation for use.

We thank Dr. Peter Bercz of our laboratory for performing the serum chemistries.

Requests for reprints should be sent to Dr. Lyman W. Condie, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

REFERENCES

1. J. J. Rook, Formation of haloforms during chlorination of natural waters, Water Treatment and Examination, 23, 234 (1974).

2. T. A. Bellar, J. J. Lichtenberg, and R. C. Kroner, The occurence of organohalides in chlorinated drinking water, J. Am. Water Works Assoc., 66, 703 (1974).

3. J. M. Symons, T. A. Bellar, J. K. Carswell, J. DeMarco, K. L. Kropp, and A. A. Stevens, National organics reconnaissance survey for halogenated organics in drinking water, J. Am. Water Assoc., 67, 634 (1975).

4. J. Doull, C. D. Klaassen, and M. O. Amdur, eds., "Casarett and Doull's Toxicology: The Basic Science of Poisons", Macmillan, New York, 1980.

5. A. E. Munson, L. E. Sain, V. M. Sanders, B. M. Kauffmann, K. L. White, D. G. Page, D. W. Barnes, and J. F. Borzelleca, Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane, Environ. Health Perspect., 46, 117 (1982).

6. C. D. Klaassen and G. L. Plaa, Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice, Toxicol. Appl. Pharmacol, 9, 139 (1966).

7. W. M. Watrous and G. L. Plaa, Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice, Toxicol. Appl. Pharmacol. 22, 528 (1972).

8. R. D. Laurie and L. W. Condie, A comparison of the renal and hepatotoxic effects of five halomethanes in mice, Pharmacologist 24, 172, Toxicologist, 2, 172 (1982).

9. R. J. Cross and J. V. Taggart, Renal tubular transport: accumulation of p-aminohippurate by rabbit kidney slices. Am. J. Physiol. 161, 181 (1950).

10. D. B. Duncan, t Tests and intervals for comparisons suggested by data, Biometrics, 31, 339 (1975).

11. J. M. Barnes and F. A. Denz, Experimental methods used in determining chronic toxicity, Pharmacol Rev., 6, 191 (1954).

12. W. M. Kluwe, Renal function tests as indicators of kidney injury in subacute toxicity studies, Toxicol. Appl. Pharmacol, 57, 414 (1981).

13. E. S. Reynolds and M. T. Moslen, Environmental liver injury: haolgenated hydrocarbons, in "Toxic Injury of the Liver Part B", E. Farber and M. M. Fisher, eds., Marcel Dekker, New York, 1980, p. 541.

☆USGPO: 1984 — 759-102/0859